

Effects of Anthranilic acid on Nickel Absorption by *Olea Europaea* Seedlings Replanted in Hydroponic Solutions

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Abstract- Hydroponic experiments were conducted to examine the effects of anthranilic acid on nickel ion (Ni^{2+}) absorption by *Olea europaea* plants. The concentration of each of Ni^{2+} and anthranilic acid in the hydroponic mixtures was varied from 0.000 to 0.025 M. Seedlings of olive (*Olea europaea*) obtained from a garden at the Department of Forestry and Wild life, Kano University of Science and Technology, Wudil and identified by Baha'uddeen Said Adam of the Department of Plant Science, Bayero University, Kano were replanted in the green house of Biological Sciences Department, Bayero University Kano. The change in plant weight (ΔWP), determined by subtracting the weight before planting from the corresponding weight after harvest decreased highly significantly ($p < 0.001$) as the concentration of Ni^{2+} was increased from 0 to 0.0250 M and anthranilic acid kept constant at 0, 0.0025 and 0.025 M respectively. The concentrations of root and shoot Ni^{2+} increased highly significantly ($p < 0.001$). The Ni^{2+} translocation factor (TF) increased highly significantly ($p < 0.001$) in the absence of anthranilic acid. As the concentration of anthranilic acid was increased to 0.0025 and 0.025 M, values of TF were less than 1 which signified increased retention of Ni^{2+} in olive (*Olea europaea*) roots with very little translocation to the shoots. Chlorophyll a and chlorophyll b contents of the olive (*Olea europaea*) seedlings first increased when the concentration of Ni^{2+} was increased from 0 to 0.0250 M in absence of anthranilic acid. But, increasing the concentration of anthranilic acid to 0.0025 and 0.025 M decreased the contents of these pigments.

Keywords- Anthranilic acid; Ni^{2+} ; *Oleaeuropaea*; Zn; Hydroponic; Proline

I. INTRODUCTION

Nickel is widely distributed in nature and is found in animals, plants, and soil (EVM, 2002). It is the 24th most abundant element, forming about 0.008% of the earth's crust (0.01% in igneous rocks). Ni received very little attention due to its dual character and complicated electronic chemistry which act as barrier to reveal the toxicity mechanism in plants (Yusuf et al., 2011). Ni is considered as a major pollutant all over the world due to the elevated quantities of Ni in soil (Echevarria et al., 1998; Faryalet al., 2007; Atiq-ur-Rehman and Iqbal, 2008). Ni has both natural and manmade sources of emission. The natural source include weathering of rocks, while many compounds of Ni like oxides, hydroxides and

acetate are released into the environment as a result of devastating human activities (Cempel and Nikel, 2006). Ni concentrations in soil are the result of smelting, burning of fossil fuels, industrial and municipal waste, vehicular emissions, mining of metals and application of Ni fertilizers (Allo-way, 1995; Salt et al., 2000). But the metallurgical and electroplating industries, chemicals used in food industry and electric batteries are major sources of Ni released into the environment (Easton, 1992).

Anthranilic acid is a white solid amino acid in pure forms whereas commercially available in yellow form. Its molecule consists of benzene ring with two adjacent functional groups, a carboxylic acid and an amine. Several investigations worked on the synthesis of anthranilic acid dyes in the various conditions which have shown significant biological activity especially against bacteria *S. aureus* and *E. coli* and. Several other mixed ligand complexes with anthranilic acid were reported to have antifungal and antibacterial potential (Raza S, Iqbal Y, Hussian I, Raza M, Shah SUA, et al. 2013).

The olive (*Olea europaea*) is a small tree, which belongs to the family *Oleaceae* and is native to tropical and warm temperate regions of the world. The tree well-known for its fruit, also called the olive, is commercially essential in the Mediterranean region as a most important source of olive oil. The fruit and the compression extracted olive have a wide range of therapeutic and culinary applications (Satish and Ansari 2013). Knowledge of the medicinal properties of the olive tree (*O. europaea*) dates back to early 1800s where it was used in liquid form as a very effective treatment against malarial infections.

II. MATERIALS AND METHODS

2.1 Plant Material

Thirty seedlings of olive (*Olea europaea*) were obtained from a garden at the Department of Forestry and Wild life, Kano University of Science and Technology, Wudil, on Monday, 17th August, 2015 at 11:00pm. The seedlings were identified by Baha'uddeen Said Adam of the Department of Plant Science, Bayero University, Kano.

2.2 Preparation of Hydroponic Solutions

2.2.1 CONTROL

The control contained $2.56 \times 10^{-6} \text{ moldm}^{-3} \text{ KNO}_3$, $5.0 \times 10^{-4} \text{ moldm}^{-3} \text{ MgSO}_4 \cdot \text{H}_2\text{O}$, $1.03 \times 10^{-3} \text{ moldm}^{-3} \text{ FeCl}_3 \cdot 6\text{H}_2\text{O}$, $2.50 \times 10^{-6} \text{ moldm}^{-3} \text{ KI}$, $2.31 \times 10^{-3} \text{ moldm}^{-3} \text{ MnSO}_4 \cdot \text{H}_2\text{O}$, $2.27 \times 10^{-3} \text{ moldm}^{-3} \text{ H}_3\text{BO}_3$, $3.57 \times 10^{-4} \text{ moldm}^{-3} \text{ Ca(NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $5.0 \times 10^{-4} \text{ moldm}^{-3} \text{ Na}_2\text{H}_2\text{P}_2\text{O}_7$ and $0.10 \text{ moldm}^{-3} \text{ HNO}_3$ 500cm³ of the control was prepared by pipetting 1.28cm³ of $0.10 \text{ moldm}^{-3} \text{ KNO}_3$, 5.15cm³ of $0.10 \text{ moldm}^{-3} \text{ FeCl}_3 \cdot 6\text{H}_2\text{O}$, 11.35cm³ of $0.10 \text{ moldm}^{-3} \text{ H}_3\text{BO}_3$, 5.00cm³ of $0.05 \text{ moldm}^{-3} \text{ MgSO}_4 \cdot \text{H}_2\text{O}$, 3.57cm³ of $0.05 \text{ moldm}^{-3} \text{ Ca(NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 5.00cm³ of $0.05 \text{ moldm}^{-3} \text{ Na}_2\text{H}_2\text{P}_2\text{O}_7$, 0.17cm³ of $0.0075 \text{ moldm}^{-3} \text{ KI}$, 23.10cm³ of $0.05 \text{ moldm}^{-3} \text{ MnSO}_4 \cdot \text{H}_2\text{O}$, and 5.00cm³ of $0.10 \text{ moldm}^{-3} \text{ HNO}_3$ into a 500cm³ volumetric flask The volume was made to mark with deionized water.

2.2.2 HYDROPONICS CONTAINING $0.0025 \text{ MOLDM}^{-3}$ AND $0.025 \text{ MOLDM}^{-3} \text{ Ni(NO}_3)_2 \cdot 6\text{H}_2\text{O}$

500cm³ of a hydroponic containing $0.0025 \text{ moldm}^{-3} \text{ Ni(NO}_3)_2 \cdot 6\text{H}_2\text{O}$ was prepared by pipetting 5cm³ of $0.25 \text{ moldm}^{-3} \text{ Ni(NO}_3)_2$ into a 500cm³ volumetric flask. The volume was made to mark with deionized water after adding other components as in the control. Similarly, hydroponics containing 0.025 moldm^{-3} nickel (II) nitrate was prepared by pipetting 50cm³ of $0.25 \text{ moldm}^{-3} \text{ Ni(NO}_3)_2$ into a 500cm³ volumetric flask. The volume was made to mark with deionized water after adding other components as in the control.

2.2.3 Hydroponics Containing $0.0025 \text{ Ni(NO}_3)_2$ and $0.0025 \text{ moldm}^{-3}$ Anthranilic acid

500cm³ of a hydroponic containing $0.0025 \text{ moldm}^{-3}$ each of $\text{Ni(NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and anthranilic acid was prepared by pipetting 5cm³ of $0.25 \text{ moldm}^{-3} \text{ Ni(NO}_3)_2$ and 5cm³ of 0.25 moldm^{-3} anthranilic acid into 500cm³ volumetric flask. The volume was made to mark with deionized water after adding the other components as in control. Other hydroponic mixtures containing different concentrations of $\text{Ni(NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and anthranilic acid were prepared by adding appropriate volumes of each of $0.25 \text{ moldm}^{-3} \text{ Ni(NO}_3)_2$ and 0.25 moldm^{-3} anthranilic acid and diluting to 500cm³ with deionised water after adding the other components as in control.

A. Replanting of Olive (*Olea Europaea*) Seedlings

Pre-treated olive (*Olea europaea*) seedlings were separately replanted in 500cm³ of hydroponics containing 0.0000, 0.0025, 0.025 $\text{moldm}^{-3} \text{ Ni(NO}_3)_2 \cdot 6\text{H}_2\text{O}$ with 0.000, 0.0025, 0.025 moldm^{-3} anthranilic acid respectively in clean 750cm³ table water plastic bottles on the 18th August, 2015 around 11:00am. Each treatment was replicated three times. The replanted seedlings were kept in the screen house of Biological Science Department, Bayero University Kano.

B. Harvesting of Seedlings

The seedlings were harvested separately harvested. Seedlings in the controls were harvested on Monday 31st, August 2015 by 1.40pm. However, seedlings in the hydroponics containing $0.0025 \text{ moldm}^{-3}$ and $0.025 \text{ moldm}^{-3} \text{ Ni(NO}_3)_2 \cdot 6\text{H}_2\text{O}$ were only harvested on 23rd, 24th and 25th August, 2015. The harvested seedlings were washed with tap water and rinsed thoroughly with deionized water and dried.

2.5 Analysis of Nickel

Two grams of each plant material was weighed in different crucibles. One milliliter of concentrated nitric acid was added and then pre-ashed by placing the crucibles on a heater until the contents turned black. The pre-ashed samples were then transferred into a muffle furnace at a temperature of 480°C for 3 hours, after which they were cooled to room temperature. The cooled samples were dissolved in 5ml of 30% hydrochloric acid and then filtered using Whatman filter papers. The filtrates were separately poured in to 50ml standard flask and made up to mark with deionized water. These were then transferred into prewashed sample bottles for analysis of the Ni^{2+} using GBC atomic absorption spectrophotometer (AOAC.1990).

2.6 Chlorophyll and Carotenoid Analysis

Chlorophyll estimation of leaves of treated and control plants was done according to the method of Arnon (1949). Two hundred milligrams (200 mg) of fresh leaf tissues of each sample was homogenized using chilled acetone in a test tubes. The homogenate was centrifuged for 10 minutes and the supernatant was collected. The residue was again extracted with 80% acetone and centrifuged. The supernatant was pooled together and the extraction process was repeated until the residue became colourless. The volume of the combined supernatant was noted. The absorbance of the solution was measured against the solvent (80% acetone) at 645 and 663 nm for chlorophyll **a** and chlorophyll **b** respectively.

2.7 PROLINE ANALYSIS

The plant material was homogenized in 3% aqueous sulfosalicylic acid (0.01g /0.5 ml) and the residue was removed by centrifugation at 3000rpm for 10 minutes. 1ml of homogenized tissue was reacted with 1ml acid-ninhydrin and 1ml glacial acetic acid in a test tube for 1 hour at 100°C and the reaction was terminated in an ice bath. The reaction mixture was extracted with 2ml toluene, mixed vigorously and left at room temperature for 30 minutes until the two phases separated. The chromophore-containing toluene (1 ml, upper phase) was warmed to room temperature and its optical activity measured at 520nm using toluene as blank. The proline concentration is determined from a standard curve using D-proline.

2.8 Data Analysis

The data were analyzed through one-way analysis of variance (ANOVA) to determine the effect of treatments Least significant difference (LSD) tests were performed to

determine the statistical significance of the differences between means of treatments.

III. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Effects of Ni^{2+} and Anthranilic acid ON THE CHANGE IN WEIGHT OF OLIVE (*Olea europaea*)

Fig.1 shows the changes in plant weight (ΔW), determined by subtracting the weights of the olive (*Olea europaea*) seedlings before replanting from the corresponding weights after harvest.

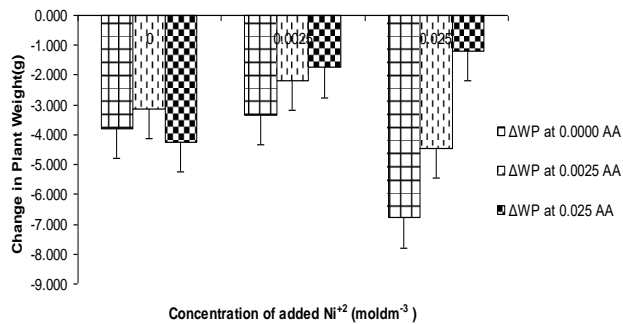


Fig.1: Change in Plant Weight against Concentration of added Ni^{2+}

Values of ΔW decreased highly significantly ($p < 0.001$) from -3.78 ± 0.212 to -6.790 ± 0.306 g, when the concentration of Ni^{2+} was increased from 0 to $0.0250 \text{ moldm}^{-3}$ in absence of anthranilic acid. Increasing the concentration of anthranilic acid to 0.0025 and 0.025 moldm^{-3} in the same concentration range for Ni^{2+} decreased the values of ΔW from 3.147 ± 0.503 to -4.470 ± 0.420 g and -4.233 ± 0.638 to -1.193 ± 0.582 g respectively.

3.1.2 Effects of Ni^{2+} and Anthranilic acid ON THE ROOT AND SHOOT UPTAKES OF OLIVE (*Olea europaea*)

Fig.2 shows the changes in root Ni^{2+} (ΔR_{tNi}) and shoot Ni^{2+} (ΔS_{hNi}) in olive (*Olea europaea*) seedlings against concentrations of added anthranilic acid (AA) in various hydroponic mixtures. The changes which were determined by subtracting the corresponding control values from the values of individual treatments increased highly significantly ($p < 0.001$).

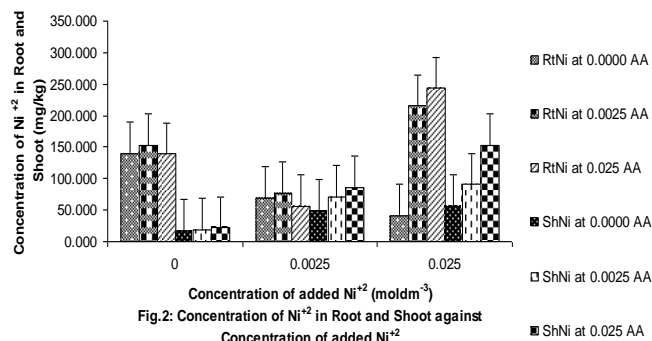


Fig.2: Concentration of Ni^{2+} in Root and Shoot against Concentration of added Ni^{2+}

As the concentration of Ni^{2+} was increased from 0 to $0.0250 \text{ moldm}^{-3}$ in absence of anthranilic acid, the root Ni^{2+} decreased from 139.889 ± 23.239 to $1.667 \pm 20.833 \text{ mg/kg}$. $0.0250 \text{ moldm}^{-3}$ in absence of anthranilic acid, the root Ni^{2+} decreased from 139.889 ± 23.239 to $1.667 \pm 20.833 \text{ mg/kg}$ and shoot Ni^{2+} increased from 17.242 ± 4.776 to $55.556 \pm 13.226 \text{ mg/kg}$ respectively. When the concentration of anthranilic acid was further increased to 0.0025 and 0.025 moldm^{-3} in the same concentration range for Ni^{2+} , increased from 152.778 ± 12.028 to $215.278 \pm 31.823 \text{ mg/kg}$ and 138.889 ± 31.823 to $243.056 \pm 31.823 \text{ mg/kg}$ respectively. The corresponding values of shoot Ni^{2+} increased from 18.333 ± 2.517 to $90.333 \pm 30.436 \text{ mg/kg}$ and 21.611 ± 1.206 to $152.778 \pm 24.056 \text{ mg/kg}$ respectively.

3.1.3 Effects of Ni^{2+} and Anthranilic acid ON Ni^{2+} TRANSLOCATION FACTOR of olive (*Olea europaea*)

Fig.3 shows the changes in Ni^{2+} translocation factor (TF) in olive (*Olea europaea*) seedlings against concentrations of added Ni^{2+} and anthranilic acid in various hydroponic mixtures. TF was determined by dividing the values of shoot Ni^{2+} (ΔS_{hNi}) with the corresponding values of root Ni^{2+} (ΔR_{tNi}).

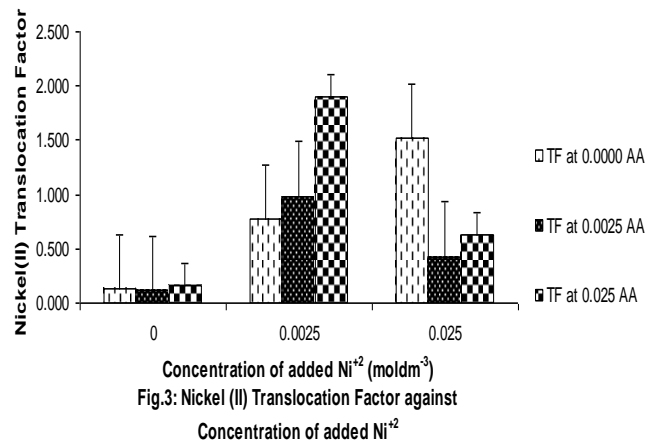
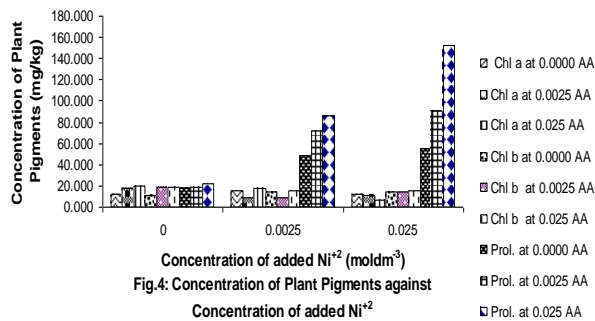


Fig.3: Nickel (II) Translocation Factor against Concentration of added Ni^{2+}

Increasing the concentration of Ni^{2+} from 0 to 0.025 in absence of anthranilic acid, increased highly significantly ($p < 0.001$) the translocation factor from 0.127 ± 0.047 to 1.515 ± 0.553 . When the concentration of anthranilic acid was increased to 0.0025 and 0.025 moldm^{-3} anthranilic acid in the same concentration range for Ni^{2+} , values of TF were less than 1 which signified increased retention of Ni^{2+} in olive (*Olea europaea*) roots with very little translocation to the shoots.

3.1.4 Effects of Ni^{2+} and Anthranilic acid ON Chlorophyll a, Chlorophyll b and Proline contents of olive (*Olea europaea*)

Fig.4 shows the changes in chlorophyll a, chlorophyll b and proline in olive (*Olea europaea*) seedlings against concentrations of added Ni^{2+} and anthranilic acid in various hydroponic mixtures.



When the concentration of Ni²⁺ was increased from 0 to 0.0250 moldm⁻³ in absence of anthranilic acid, chlorophyll **a** and chlorophyll **b** increased from 11.753±0.008 to 12.513±0.001 and 10.783±0.027 to 14.323±0.134mg/kg respectively. The proline content first increased from 21.602±0.087 to 113.810±0.043 and then decreased to 91.111±0.164mg/kg. When the concentration of anthranilic acid was increased to 0.0025 moldm⁻³ in the same concentration range for Ni²⁺ the chlorophyll **a** and chlorophyll **b** contents decreased highly significantly ($p < 0.001$) from 18.041±0.004 to 10.514±0.007 and 10.514±0.007 to 13.984±0.002mg/kg respectively. However, the proline content increased from 34.545±0.189 to 56.263±0.066mg/kg. When the concentration of anthranilic acid was further increased to 0.0250 moldm⁻³, chlorophyll **a** and chlorophyll **b** contents decreased from 20.387±0.003 to 6.194±0.015 and 19.143±0.012 to 15.298±0.003mg/kg respectively. But, the proline content increased from 41.732±0.043 to 46.797±0.075mg/kg respectively.

3.2 Discussion

Earlier studies have shown that excessive nickel can inhibit seed germination, plant growth, mitotic activities (Madhava Rao & Sreetsy, 2000), induced chlorophyll degradation, chlorosis, necrosis and wilting which interfered with photosystem activity (Leon *et al.* 2005).

In this work, a general decrease in plant weight was observed in all treatments including the control. However, Duman and Ozturk, (2009) reported an increase in biomass in plants exposed to 1 mg/L Ni. In comparison with the control they observed a significant decrease in biomass only at 25mg/L Ni for 7 days.

The most important elemental uptake in plants is in the root (Sharma and Gaur, 1995). High amount of nickel can be rapidly taken up and accumulated by the plant root system (Ali *et al.*, 2008; Aksoyet *et al.*, 2005; Chooet *et al.*, 2006, Dumanet *et al.*, 2007). As reported by Kov'ačiket *et al.*, (2009), Madhava and Sresty (2000) the uptake of nickel by plant was concentration dependent.

The extent and rate of translocation of metals within plants depended on metal and plant species (Deng *et al.*, 2004). The figure for the translocation factor indicates that the accumulated Ni²⁺ was largely retained in the roots (TF ratio <

1). These results suggest that protective barriers exist to prevent the penetration of Ni²⁺ from the roots to the shoots (Hozhinaet *al.*, 2001). Yang *et al.*, (1996) stated that high nickel concentrations would cause plant growth to be weak which leads to depression, metabolic disorders and chlorosis. In this research, it was found that as the concentrations of Ni²⁺, anthranilic acid and the duration of exposure increased, there was a decline in growth rate and biomass, a result consistent with the findings of Kabala *et al.*, (2008). This may be due to the degradation of cell membrane and wall induced by oxidative stress cause by absorbed nickel (Singh *et al.*, 2006; Sinha *et al.*, 2005).

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